ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

# CK1δ Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

## Scientific Background:

CK1δ is a member of the CK1 family of serine/threonine protein kinases which play an important role in diverse cell processes, including DNA replication and repair. CK1δ is a regulator of Yes-associated protein (YAP) transcription coactivator which is a key regulator of organ size and a candidate human oncogene. CK1δ is activated by CCK2R and this then phosphorylates PKD2 at Ser244. Phosphorylation of PKD2 leads to its nuclear accumulation and efficient phosphorylation of nuclear PKD2 substrates in human gastric cancer cells (1). CKIδ can phosphorylate in vitro deoxycytidine kinase (dCK) which is a key enzyme in the salvage of deoxynucleosides. Phosphorylation of dCK by CKIδ in vitro correlates with increased activity of this enzyme (2).

1. von Blume J. et al: Phosphorylation at Ser244 by CK1 determines nuclear localization and substrate targeting of PKD2. EMBO J. 2007 Nov 14;26(22):4619-33.

2. Smal, C. et al: Casein kinase 1delta activates human recombinant deoxycytidine kinase by Ser-74 phosphorylation, but is not involved in the in vivo regulation of its activity. Arch Biochem Biophys. 2010 Oct 1;502(1):44-52.

## ADP-Glo™ Kinase Assay

### Description

ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase (Fig. 1). The luminescent signal positively correlates with ADP amount (Fig. 2) and kinase activity (Fig. 3A). The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling (Fig. 3B). The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.

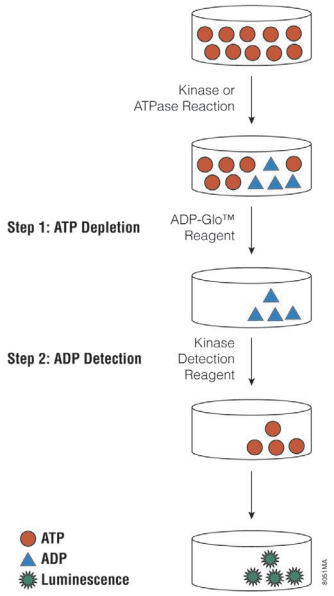


**Figure 1. Principle of the ADP-Glo™ Kinase Assay.** The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

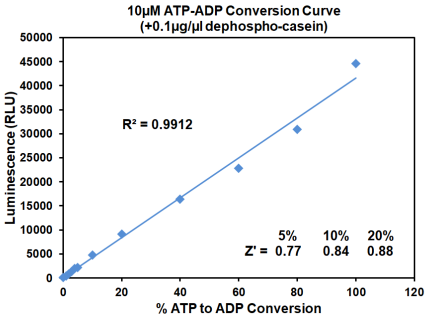


**Figure 2. Linearity of the ADP-Glo Kinase Assay.** ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

# ADP-Glo™ Kinase Assay Application Note
# Ser/Thr Kinase Series

**The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol**

## Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction buffer.
- Add to the wells of 384 low volume plate:
  - ✓ 1 µl of inhibitor or (5% DMSO)
  - ✓ 2 µl of enzyme (defined from table 1)
  - ✓ 2 µl of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5 µl of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10 µl of Kinase Detection Reagent.
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1 second).

**Table 1. Enzyme Titration.** Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

| Enzyme, ng | 200 | 100 | 50 | 25 | 12.50 | 6.25 | 3.13 | 1.56 | 0.78 | 0.39 | 0.20 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Luminescence | 57,295 | 46,505 | 36,905 | 25,662 | 17,096 | 10,354 | 6,295 | 2,952 | 1,479 | 856 | 634 | 326 |
| S/B | 176 | 143 | 113 | 79 | 52 | 32 | 19 | 9 | 5 | 3 | 2 | 1 |
| % Conversion | 138 | 112 | 89 | 62 | 41 | 25 | 15 | 7 | 3 | 2 | 1 | 0 |

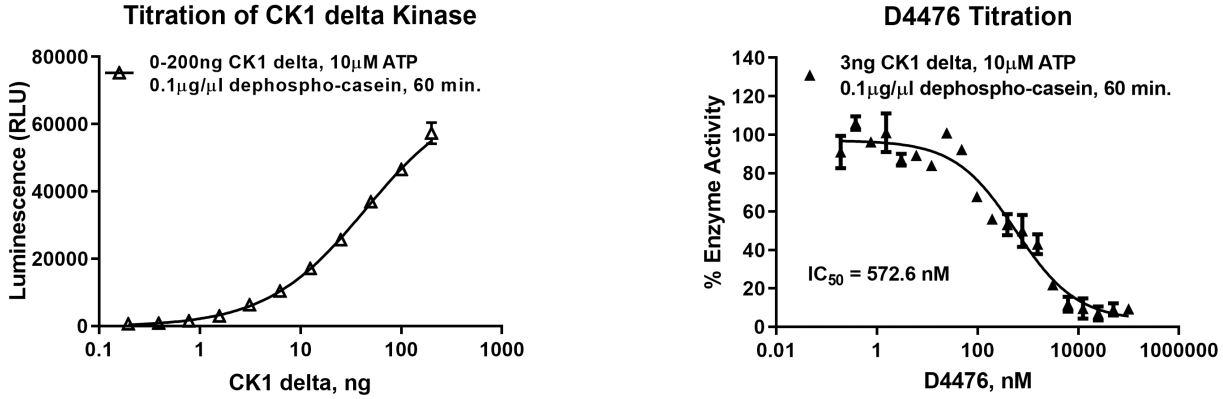


**Figure 3. CK1δ Kinase Assay Development.** (A) CK1δ enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3ng of CK1δ to determine the potency of the inhibitor ($IC_{50}$).

## Ordering Information:

| Products | Size | Cat. # |
|---|---|---|
| CK1δ Kinase Enzyme System | 10µg | VA7405 |
| | 1mg | VA7406 |
| ADP-Glo™ + CK1δ Kinase Enzyme System | 1 Each | VA7407 |